

REMARKS

Preliminary Remarks

Reconsideration and allowance of the present application based on the following remarks are respectfully requested. Claims 1-15 are currently pending in this application and remain at issue.

In paragraph 1 of the official action, the examiner acknowledged the claim of foreign priority under 35 U.S.C. §119(a)-(d) to Germany Patent Application No. 19912384.5 filed on March 19, 1999. The applicants submit herewith a certified copy of this application.

In paragraph 3 of the official action, the examiner objected to the phrase “nucleotide sequence coding for said malate:quinone oxidoreductase” in claim 5 for lacking clarity. Claim 5 has been canceled without prejudice and therefore the objection to claim 5 is moot.

The applicants do not intend by these or any amendments to abandon subject matter of the claims as originally filed or later presented, and reserve the right to pursue such subject matter in continuing applications.

Patentability Remarks

Rejection Under 35 U.S.C. §112, Second Paragraph

In paragraphs 5-9 of the official action, the examiner variously rejected claims 2-15 under 35 U.S.C. §112, second paragraph, for allegedly being indefinite. Specifically, the examiner alleged the phrases “the improvement of claim,” “the improvement of any one of claims,” and the “improvement according to” in claims 2-10 are indefinite and suggested amending these phrases to “the process of claim...wherein.” The examiner also asserted the claims 8 and 15 were indefinite for the recitation of “production of L-lysine” since there is no Markush group in claims 7 and 14 from which claims 8 and 15 depend, respectively. The examiner alleged that claim 9 was indefinite for the recitation of “the gene coding for dihydrodipicolinate synthase” since there was no antecedent basis for the gene. The examiner concluded by stating claim 11 was indefinite for the recitation of “fermenting the bacteria produced in step a” because step (a) refers to the amplification of a malate:quinone oxidoreductase gene in bacteria and not the production of bacteria.

Solely for the purpose of expediting prosecution, and without prejudice to the applicants right to seek broader claims in a continuing application, the applicants have

canceled claims 2-10 without prejudice. Further, the applicants note that new claims 16-23 do not contain the language referred to by the examiner. In view of the foregoing amendments, the applicants submit that the rejection of claims 2-10 pursuant to 35 U.S.C. §112, second paragraph, for indefiniteness, should be withdrawn and should not be extended to new claims 16-23.

Rejections Under 35 U.S.C. §112, First Paragraph, Written Description

In paragraph 11 of the official action, the examiner rejected claims 1-15 under 35 U.S.C. §112, first paragraph, for allegedly failing to comply with the written description requirement. Specifically, the examiner alleged that while the specification discloses the production of L-lysine and L-threonine by transforming *C. glutamicum* with a plasmid containing the malate:quinone oxidoreductase (*mgo*) of *C. glutamicum* strain ATCC 13032, the specification does not describe (1) malate:quinone oxidoreductase gene from other sources or other coryneform bacteria, (2) the production of other L-amino acids as claimed, (3) methods to enhance the intracellular activity of malate:quinone oxidoreductase, such as structurally modifying the coding region of the corresponding mpo genes, (4) methods to identify or enhance or eliminate particular genes encoding particular enzymes of any coryneform bacteria wherein said enzyme is associated with the biosynthetic pathway of any L-amino acid, (5) the structure of other genes encoding dihydrodipicolinate synthases, and (6) the structure of other DNA fragments associated with S-(2-aminoethyl)-cysteine resistance. The examiner concludes by stating one of skill in the art cannot reasonably conclude that the applicants had possession of the claimed invention at the time the instant application was filed.

As stated above, claims 1-15 have been canceled without prejudice thereby rendering moot the rejection as applied to each of these claims.

The applicants submit that this rejection should not be extended to the new claims for the following reasons. New claim 16 is directed to a fermentation process for the preparation of a desired L-amino acid selected from the group consisting of L-aspartic acid, L-asparagine, L-homoserine, L-threonine, L-isoleucine, L-lysine, and L-methionine, wherein the following steps are carried out: (a) fermentation of an *Corynebacterium* or *Brevibacterium* strain in a fermentation broth for producing the desired L-amino acid, wherein a gene encoding malate:quinone oxidoreductase (*mgo*) of *Corynebacterium glutamicum* strain ATCC 13032 is overexpressed, (b) concentration of the fermentation broth to eliminate water and increase the

concentration said L-amino acids in the broth and *Corynebacterium*, and (c) isolation of the L-amino acid from the fermentation broth and *Corynebacterium* of step (b). Support for new claim 16 can be found throughout the specification, for example, page 3, line 31 to page 4, line 19; page 7, lines 21-27; and Examples 1, 2 and 4. Specifically, Example 1 teaches the transformation of a *Corynebacterium* strain with pRM17, which can overexpress the full length gene encoding for malate:quinone oxidoreductase of *C. glutamicum*. The applicants also specifically teach the claimed process of overexpressing the gene encoding for malate:quinone oxidoreductase lead to an increase production in not only L-lysine and L-threonine (as discussed in Examples 2 and 4), but also can be specifically applied to the production of the L-amino acids L-aspartic acid, L-asparagine, L-homoserine, L-isoleucine, and L-methionine (see page 7, lines 21-26). The applicants respectfully submit also that the claimed process is adequately describe for application in other *Corynebacterium* strains (besides *C. glutamicum*) and *Brevibacterium* strains and lists these specific industrial strains on page 4 of the specification.

New claim 19 is directed to a fermentation process for the preparation of a desired L-amino acid selected from the group consisting of L-lysine, and L-methionine, wherein the following steps are carried out: (a) fermentation of an *Corynebacterium glutamicum* strain in a fermentation broth for producing the desired L-amino acid, wherein a gene encoding malate:quinone oxidoreductase (*mpo*) of *Corynebacterium glutamicum* strain ATCC 13032 is overexpressed, (b) concentration of the fermentation broth to eliminate water and increase the concentration said L-amino acids in the broth and *Corynebacterium glutamicum* , and (c)isolation of the L-amino acid from the fermentation broth and *Corynebacterium glutamicum* of step (b). Support for new claim 19 is found throughout the specification, for example, Examples 1-4. In the official action, the examiner acknowledged the specification discloses the production of L-lysine and L-threonine by transforming *C. glutamicum* with a plasmid containing the *mgo* gene of *C. glutamicum* strain ATCC13032 (see page 5).

The applicants also respectfully submit new claims 22 and 23 are fully supported by the teachings of the specification. Specifically, claim 22 is directed to a fermentation process for the preparation L-lysine, wherein the following steps are carried out: (a) fermentation of an *Corynebacterium glutamicum* strain in a fermentation broth for producing L-lysine, wherein a gene encoding malate:quinone oxidoreductase (*mpo*) of *Corynebacterium glutamicum* strain ATCC 13032 is overexpressed, (b) concentration of the fermentation broth to eliminate water and increase the concentration said L-lysine in the broth and

Corynebacterium glutamicum, and (c) isolation of the L-amino acid from the fermentation broth and *Corynebacterium glutamicum* of step (b). New claim 23 is directed to the process according to claim 22, further comprising overexpressing one or more genes selected from the group consisting of a *dapA* gene encoding for dihydrodipicolinate synthase of *Corynebacterium glutamicum* and a gene encoding for S-(2-aminoethyl)-cysteine resistance. The applicants submit the specification clearly describes overexpression of the full-length *mgo* gene increases the production of L-amino acids in the Examples. The specification also teaches the overexpression of the *dapA* gene coding for dihydrodipicolinate synthase and the gene encoding S-(2-aminoethyl)-cystein resistance have also been shown to increase L-lysine production. Accordingly, these combined teachings of the specification fully support claims 22 and 23.

In view of the foregoing amendment and remarks, the applicants submit that the rejection of claims 1-15 pursuant to 35 U.S.C. §112, first paragraph, for lack of written description, is moot, and a rejection of new claims 16-23 on the same grounds would be improper.

Rejection Under 35 U.S.C. §112, First Paragraph, Enablement

Claims 6 and 13

In paragraph 12 of the official action, the examiner rejected claims 6 and 13 under 35 U.S.C. §112, first paragraph, for allegedly lacking enablement. Specifically, the examiner stated that while the novel vector has been deposited under the Budapest Treaty there is no indication in the specification as to the public availability (see attached Appendix).

Claims 6 and 13 have been replaced without prejudice by claims 18 and 21. The applicants submit herewith a declaration of biological deposit executed by the undersigned. Accordingly, the applicants submit that the rejection of claims 6 and 13 is moot, and a rejection of new claims 18 and 21 on the same grounds would be improper.

Claims 1-15-Part 1-Operability

In paragraph 13 of the official action, the examiner rejected claims 1-15 under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with "enablement." Specifically, the examiner alleged that co-pending application 10/118,325 (common assignee Degussa

AG; U.S. Patent Publication No. 20030044943) teaches that attenuation of the *C. glutamicum mgo* gene in *C. glutamicum* results in the increased production of L-amino acids, such as L-lysine. The examiner concludes that in view of the conflicting and opposing teachings of both specifications (attenuation in 10/118,325 vs. overexpression in the present application), and the alleged lack of additional information in the instant application, it is unclear as to whether the method disclosed in the instant application is operable because one cannot reasonably conclude the applicants have provided sufficient guidance to enable the claimed invention. In view of the foregoing amendments and remarks, the applicants respectfully traverse this rejection.

The applicants respectfully submit that the specification provides clear operability and guidance through its working examples to practice the amended claims. Specifically, Tables 1 and 2 clearly demonstrate that when the full length *mgo* gene is overexpressed via the pRM17 plasmid in *C. glutamicum* strain DSM5715, the levels of L-lysine and L-threonine are 1.4 g/l and .18 g/l higher than in DSM5715 alone. These results unequivocally demonstrate that by overexpressing malate:quinone reductase increases L-amino production.

Furthermore, the applicants directly correlate the expression of the full length *mgo* gene as the reason for increased L-amino acid production by comparing the effects of DSM5715 transformed with either pJC1 or pRM17. Plasmid pRM17 contains the full length *mgo* gene and allows this gene to be overexpressed. The base shuttle vector of pRM17 is pJC1. Plasmid pJC1 **does not harbor** the *mgo* gene. Table 1 demonstrates that DSM5715 transformed with pJC1 produces 16.5 g/l of L-lysine. DSM5715 transformed with pRM17 (full length *mgo* gene) produces 17.8 g/l. Nearly every genetic and biological component is the same between DSM5715::pJC1 and DSM5715::pRM17 (same base vector, same host cell, same fermentation conditions) except for the fact DSM5715::pRM17 contains the full length *mgo* gene. Accordingly, the full length *mgo* gene is the direct cause for the 1.3 g/l increase of L-lysine production.

In view of the examiner's comments, it appears to the applicants that the examiner is questioning the veracity of the results of Tables 1 and 2. Yet, the applicants submit the enablement requirement simply requires sufficient information to inform those skilled in the relevant art how to both make and use the claimed invention. The inventors, through their inventors declarations, have attested that their results demonstrating that the overexpression of the full length *mgo* gene causes an increase in the levels of L-amino acids are both

accurate and true. It is irrelevant that co-pending application 10/118,325 discloses an attenuated *mgo* gene that increases the production of L-lysine. Clearly the teachings of the applicants disclosure **of this application** (*i.e.*, cloning the *mgo* gene from *C. glutamicum* in a high expression vector like pRM17, transforming the vector into a Brevibacterium or Corynebacterium strain like *C. glutamicum*, fermenting these transformed isolates, and measuring the levels of L-amino acids like L-lysine and L-threonine) are operable to enable one of skill in the art to practice the claimed invention. Accordingly, the applicants respectfully submit the examiner has improperly rejected claims 1-15 as being non-enabled.

Claims 1-15-Part II-Undue Experimentation

In paragraph 14 of the official action, the examiner further rejected claims 1-15 under 35 U.S.C. § 112, first paragraph, for lacking enablement. Specifically, the examiner alleged the specification does not provide enablement for a (1) method for producing any L-amino acids by cultivating coryneform bacteria which have been modified in any way to enhance the intracellular activity of any malate::quinone oxidoreductase; (2) a method as described in (1) wherein the coryneform bacteria have been further modified in any way to enhance the activity of any enzyme from any organism associated with the biosynthetic pathway of any L-amino acid; or (3) the method as described in (1) wherein the coryneform bacteria has been further modified in any way to eliminate any metabolic pathway which reduces the formation of any L-amino acid.

Solely to expedite prosecution, and without prejudice to seeking broader claims in a continuing application, the applicants have canceled claims 1-15 and have introduced new claims 16-23. The examiner acknowledged that the specification is enabled for a method for producing L-lysine or L-threonine in *C. glutamicum* by cultivating *C. glutamicum* which has been transformed with a plasmid comprising the *C. glutamicum* malate:quinone oxidoreductase gene such that *mgo* gene is overexpressed. The applicants further submit that the specification fully enables one of skill in the art to transform other Corynebacterium and Brevibacterium strains with the *C. glutamicum mgo* gene to overproduce not only L-lysine and L-threonine (discussed in the working examples), but also L-aspartic acid, L-asparagine, L-homoserine, L-isoleucine, and L-methionine (see page 7, lines 19-27 and page 4, lines 4-19). Because there is a sufficient number of detailed procedural examples demonstrating how to transform specific Corynebacterium strains to overproduce L-lysine and L-threonine, the remaining description sufficiently enables one of skill in the art to apply the teachings to

Corynebacterium and Brevibacterium strains to produce particular L-amino acids (L-aspartic acid, L-asparagine, L-homoserine, L-isoleucine, and L-methionine) and practice the claimed inventions of new claims 16-23. Accordingly, in view of the foregoing amendments and remarks, the applicants respectfully submit that the rejection of claims 1-15 under 35 U.S.C. §112, first paragraph is moot, and a rejection of new claims 16-23 on the same grounds would be improper.

Rejection Under Nonstatutory Double Patenting

In paragraphs 16-22 of the official action, the examiner rejected claims 1, 2, 4, 5, 11, and 15 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over various claims in co-pending U.S. Application Nos. 10/178219, 10/375355, 09/804073, 09/770688, 09/938688, 09/938540, 09/733386, and 09/796431. Specifically, claims 1, 2, 4, 5, 11, and 15 are allegedly obvious in view of claim 10 of co-pending application no. 10/178219, claim 14 of co-pending application no. 10/375355, claim 15 of co-pending application no. 09/804073, claim 14 of co-pending application no. 09/770688, claim 18 of co-pending application no. 09/938540, claims 16 and 17 of co-pending application 09/733386, and claim 24 of co-pending application no. 09/796431. The examiner asserted each of conflicting claims are directed to a fermentation process for the production of L-amino acid which uses a coryneform microorganism modified by containing a particular gene (for example, 09/938,540 is directed to catabolite control protein (ccpA1)), wherein the expression of said gene has been reduced or eliminated in combination with a malate::quinone oxidoreductase gene (*mgo*) that has been enhanced. The examiner further alleged that claims 1, 2, 4, 5, 11, and 15 are each directed at least to a process of producing an L-amino acid with a modified coryneform microorganism such that the activity of malate::quinone oxidoreductase has been overexpressed. Accordingly, the examiner asserted the claims in the copending applications would anticipate claims 1, 2, 4, 5, 11, and 15 of the instant application.

The applicants respectfully submit the obviousness double patenting rejection of claims 1, 2, 4, 5, 11, and 15 in view of claim 15 of co-pending application no. 09/804073, claim 14 of co-pending application no. 09/770688, and claims 16 and 17 of co-pending application no. 09/733386 are moot in view of the fact that co-pending application nos. 09/804073, 09/770688, and 09/733386 are abandoned (see exhibit A). Accordingly, the rejection based upon these applications should be withdrawn.

With respect to the obviousness double patenting rejection of claims 1, 2, 4, 5, 11, and 15 in view claim 10 of co-pending application no. 10/178219, claim 14 of co-pending application no. 10/375355, claim 18 of co-pending application no. 09/938540, and claim 24 of co-pending application no. 09/796431, the applicants enclose herewith a terminal disclaimer, executed by the undersigned on behalf of the applicants.


In view of the foregoing comments and submitted declarations, the applicants respectfully submit that the rejection of claims 1, 2, 4, 5, 11, and 15 under non-statutory obviousness double patenting has been overcome and should be withdrawn.

CONCLUSION

In view of the foregoing, the claims are now believed to be in form for allowance, and such action such action is hereby solicited. If any point remains in issue which the examiner feels may be best resolved through a personal or telephone interview, please contact the undersigned at the telephone number listed below.

Respectfully submitted,

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